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Ontogenetic shift in *Daphnia*-algae interaction strength altered by stressors: revisiting Jensen's inequality

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Abstract Interaction strength among species plays a crucial role in shaping the functioning of ecological communities, but it is often assumed to be insensitive to inter-individual variation in underlying parameters such as attack rates or handling time. Ecological factors including stressors exert age/size-dependent effects on such behavioral parameters, promoting shifts in the distribution of parameter values over ages. Here we analyze the effects of the pesticide methamidophos on the *Daphnia*-microalga interaction strength. We first analyze age-dependent effects of the pesticide on the *Daphnia* functional response, and then decompose the population-level effects of the stressor into contributions of shifts in elevation (i.e., vertical effect) versus shifts in nonlinearity (i.e., nonlinear effect) of the response of interaction strength over consumer age. Our results show that (1) Rogers and Holling type II functional response models best fitted the empirical functional responses of *Daphnia* of different ages, (2) attack rate and handling time were affected by the pesticide, (3) these effects were age-specific, shifting the average attack rate and both the mean and coefficient of variation of handling time of different age classes, (4) population level interaction strength was affected by pesticide exposure by variation in both elevation and nonlinearity of its response over consumer age. We show that both vertical and nonlinear effects were important in magnitude but opposite in sign. The consequences of factors that exert age/size dependent effects can only be evaluated through properly considering inter-individual variation.

Keywords Functional response · Structured populations · Nonlinear functions · Feeding rate · Ecotoxicology

Introduction

The strength of species interactions can have large consequences on community dynamics and stability (Berlow et al. 2004; Vázquez et al. 2015), with strong interactions tending to destabilize population and community dynamics (McCann et al. 1998). Interaction strength of predators on their prey is measured commonly as the effect of predator species on the per capita population growth rate of prey (Laska and Wootton 1998; Berlow et al. 2004; Wootton and Emmerson 2005). Considering only lethal effects (simplistic but often assumed in most theoretical models of predator–prey interaction), interaction strength of predators on their victims is often described by the per capita killing rate of prey, which is governed by the prey availability as well as by interaction parameters such as attack rate and handling time, which depend on properties and traits of both consumer and resource (Pawar et al. 2015). This relationship is known as the predator's functional response. However, the population functional response, that is known to affect community dynamics, is determined by the aggregation (i.e., averaging) of individual functional responses (Bolnick et al. 2011), which often exhibit considerable variation. In age-structured populations, individual traits vary considerably due to ontogenetic differences among organisms (Polis 1984). In many animal populations younger individuals are smaller and have both lesser energy requirements and lesser capability to gather food and consequently exhibit reduced attack rates and/or increased handling time of prey items. Variation in these behavioral parameters within populations can cause a significant shift in community and ecosystem dynamics (Okuyama 2008; Miller and Rudolf 2011; Schreiber et al. 2011; Violle et al. 2012; Persson and De Roos 2013; Rudolf and Rasmussen

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2013; Gibert and Brassil 2014; Gibert et al. 2015; Hughes et al. 2015).

Many environmental factors such as temperature, chemical composition of the medium, interaction with other organisms, as well as anthropogenic stressors, affect the parameters that determine interaction strength in different mode and magnitude according to the age of individuals. For example, individuals of different age exhibit different tolerance to pesticide exposure (Day and Kaushik 1987; Hanazato 1991; Takahashi and Hanazato 2007). Given that external pressures such as climatic factors or pollution are not generally expected to affect functional response parameters in equal magnitude in individuals of different age, the outcome of these effects on interaction strength at the population level is difficult to predict.

In this study we analyze the effects of a model stressor, the pesticide methamidophos, on the *Daphnia*-microalga interaction strength. Specifically, we study the shift caused by methamidophos exposure on the ontogenetic variation of parameters that determine interaction strength, namely predator attack rate and handling time. Finally, we decompose the effect of methamidophos on population-level maximal interaction strength into contributions of (1) magnitude of responses in individuals of average age, namely vertical effects, and (2) degree of nonlinearity of the relation between interaction strength and consumer age, namely nonlinear effects.

Methods

Test organisms and stressor

We constructed empirical functional responses of the microcrustacean herbivore *Daphnia ambigua* feeding on the green microalga *Pseudokirchneriella subcapitata*. Individuals of *D. ambigua* were collected at Peñuelas reservoir, Valparaíso, Chile (33°07'S, 71°24'W) and a stock was maintained in the laboratory at 20 ± 1 °C and photoperiod 16:8 (L:D). Individuals were grown in COMBO medium (Kilham et al. 1998) and fed every 2 days with 10^6 cells mL⁻¹ algal suspension.

Methamidophos (O,S-dimethyl phosphoramidothioate) is used to control pests in many crops (WHO 1993; Malato et al. 1999). This pesticide has high water solubility; its residues and fate in the environment are of concern (Yen et al. 2000). This organophosphate is one of the most toxic and broad-spectrum acetylcholinesterase inhibitors (Hussain 1987). It is highly toxic to aquatic animals, including fish (Tomin 1994), decapods (García de la Parra et al. 2006) and *Daphnia magna* (Lin et al. 2006). Following OECD (2004), we assessed methamidophos 24 h-LC50 (i.e., stressor concentration that causes 50 % mortality in 24 h) for the test organisms under the same environmental conditions used in the main experiments. We obtained a 24 h-LC50

value of 369 µg L⁻¹. After performing preliminary experiments and in order to obtain measurable effects on feeding rates without *Daphnia* mortality, we selected an exposure time of 6 h to methamidophos at the assessed 24 h-LC50 value. After exposing the individuals, feeding rates were measured in clean medium.

Feeding rate experiments

We assessed the ontogenetic changes in feeding rates of the herbivore *D. ambigua* over a gradient of food density, both with and without prior exposure of consumers to the pesticide methamidophos. In this way we can analyze how *Daphnia*'s functional responses change as a function of age and pesticide exposure. We use here the common definition of functional response (e.g., Jeschke et al. 2002): the relationship between feeding rate (i.e., number of prey eaten per predator per unit time) and prey density. We measured feeding rates of the herbivore at various ages: 1 day old (neonates), 3 and 5 days old (juveniles) and adults of age 7 and 9 days. Neonates of the same cohort (<24 h old) were extracted from the third clutch of mothers that were isolated from the stock culture. These neonates were grown until they reached the required age to be used in the experiments. The test organisms were grown in 5 L aquaria and fed every 2 days with a suspension of *P. subcapitata* at 10^6 cells mL⁻¹. The experiments were not conducted simultaneously, but within a period of 9 days. On each of the days when the test organisms reached each of the experimental ages, a group of them was randomly chosen and assigned to one of two treatments: (1) pesticide exposure (T treatments) and (2) controls without pesticide exposure (C treatments). In the T treatments, individuals were exposed for 6 h to methamidophos at a concentration of 369 µg L⁻¹, as explained above. During this period individuals in both T and C treatments were maintained in COMBO medium at a density of 30 ind L⁻¹ and fed with 10^6 cells mL⁻¹ of *P. subcapitata*. After exposure individuals were gently washed in clean media and a random subset from both T and C treatments was transferred to 1 mL vessels containing different food levels: 10^5 , 5×10^5 , 2×10^6 , 4×10^6 and 6×10^6 cells mL⁻¹ of *P. subcapitata*. We used four replicates in each of the 5×2 treatments. After an acclimation of 1 h we started foraging trials by introducing the predators into prey dishes at each of the experimental food levels, whose depletion we attempted to minimize during the test. The experiments were conducted in darkness at 20 °C in an orbital shaker incubator at a slow agitation rate (100 rpm) to prevent algal settling. Due to considerable differences in feeding rate over consumer age, the duration of experiments, volume of the vessels and number of individuals per experimental unit were adjusted for each age class in order to obtain reliable measurements. In particular, we set the experimental conditions to fulfill two basic criteria: (1) minimize the

Table 1 Summary of the experimental setup for each experimental unit at each age of the test organisms

Age (day)	No. of ind. per experimental unit	Test duration (h)	Test volume (mL)
1	4	4	0.5
3	2	4	0.5
5	2	4	1
7	2	4	1
9	1	2	1

This set was applied for all combinations of five food levels and two levels of pesticide exposure, with four replicates each. In total, 200 experimental units were used

experimental time, to avoid indirect mechanisms of consumer dependence and excessive algal depletion (Fussmann et al. 2005), and (2) maintain the total body surface of consumers (i.e., the sum over individuals in a vessel) approximately constant per unit volume in each experimental unit. This was done as a way of controlling the clearance rate of algae over consumer ages, assuming that clearance rate is proportional to body surface (Kooijman and Metz 1984). Consumer body surface per volume was $9.1 \pm 1.9 \text{ mm}^2 \text{ mL}^{-1}$ (mean and standard deviation). A summary of the chosen experimental conditions is shown in Table 1. To determine feeding rate, the density of algal cells before and after the addition of *D. ambigua* consumers was estimated under a microscope in a hemocytometer (Neubauer chamber). We used a standard equation (Peters 1984) to calculate per capita feeding rates, $f = V(C_0 - C_t)N^{-1}t^{-1}$, where C_0 and C_t (cells mL^{-1}) are initial and final cell concentration, V (mL) is the volume of the container, N is the number of animals in the container and t (h) is the length of time the animals were allowed to feed. In addition, we measured the variation in algal concentration in each combination of food level and consumer age, but without consumers. We used two replicated flasks in each of these treatments. This allowed us to check for possible changes in algal abundance due to reasons other than feeding and correct for this effect. Nevertheless, such corrections were not needed since there were no changes in cell concentration in these assays.

Statistics

Feeding rate experiments

The effects of pesticide exposure, consumer age and food density on the feeding rate of *D. ambigua* were assessed using a generalized linear model analysis with a negative binomial error distribution. We selected the functional response model that best described the variation in feeding rate with food concentration. For this we used nonlinear regression to fit raw feeding rate values versus food concentration at each age to a number of functional response models (Table 2) for both pesticide and control treatments. Model selection was done using the

Table 2 Set of alternative functional response models to be tested against our experimental data

Model	Type
$y = \frac{ax}{1 + ahx}$	Standard Holling type II
$y = \frac{ax^2}{1 + ahx^2}$	Holling type III
$y = x(1 - e^{a(hy-T)})$	Rogers random-predator
$y = \frac{ax^\theta}{1 + ahx^\theta}$	Generalized holling type III
$y = \frac{ax}{1 + ahx^\theta}$	Holling type IV
$y = \frac{ax^\gamma}{1 + ahx^\theta}$	Generalized Holling

Variables and parameters are: number of prey eaten (y), number of prey present at the start of the experiment (x), attack rate (a), handling time (h), total time of the experiment (T) and shape coefficients (θ, γ)

corrected Akaike information criterion (AICc, Burnham and Anderson 2004). Individual values of AICc were scaled to $\Delta\text{AICc} = \text{AICc} - \text{AICc}_{\min}$, where AICc_{\min} is the minimum value of AICc computed for the different models. This transformation forces the best model to have a $\Delta\text{AICc} = 0$ and greater values for the other models.

Predator-prey interaction strength

As stated above, we used the feeding rate of prey (algae) by an individual predator (*Daphnia*) as the measure of interaction strength (Yodzis 1989; Wootton and Emmerson 2005). This measure corresponds to elements of the Jacobian matrix, which does not need to be evaluated at equilibrium (Wootton 1997). Since this measure depends on prey availability, we use hereafter the maximal interaction strength, defined as the limit of the killing rate as prey availability approaches infinity. The existence of this value is assured in saturating models of functional responses such as the ones used here (Table 2). In particular, the maximal interaction strength in our models is given by the inverse of handling time, h^{-1} . This approach to measure interaction strength is common in the ecological literature (Sala and Graham 2002; Berlow et al. 2004), although not free of criticism (Abrams 2001).

Bootstrapping

Ninety-five percent confidence intervals for mean values of the functional response parameters were obtained by bootstrap using the percentile method (Manly 2007).

For each food level in a given consumer age, four feeding rate values were randomly sampled with

replacement. This new dataset was fitted to the selected functional response model to obtain the parameter values. This procedure was repeated 1500 times for each consumer age. From the set of 1500 parameter values obtained by this method, we calculated the 95 % confidence intervals of their mean values as the interval within percentiles 2.5 and 97.5.

Nonlinearity, Jensen's inequality and decomposition of pesticide effects

A property of nonlinear functions called Jensen's inequality (Jensen 1906; Ruel and Ayres 1999) states that the average value of a function $f(x)$ is different from the function evaluated at the average value of x . For accelerating functions (i.e. with a positive second derivative), the average result of $f(x)$, $\overline{f(x)}$, is greater than the function evaluated at the average value of x , $f(\bar{x})$. Conversely, for decelerating (i.e. with negative second derivative) functions, $\overline{f(x)}$ is smaller than $f(\bar{x})$. The consequence is that the variance of x consistently increases (in accelerating functions) or decreases (in decelerating functions) the averaged dependent variable $\overline{f(x)}$. Consequently, all other things being equal the magnitude of the difference between $\overline{f(x)}$ and $f(\bar{x})$ increases with the degree of nonlinearity of the function.

For our purposes we are interested in variations in population maximal interaction strength $\overline{\psi(x)}$ driven by an external pressure (pesticide exposure) exerted on individuals of different ages. If the pesticide exposure alters the biological response of individuals homogeneously over ages, then the population effect is explained entirely by a change in the height of the $\psi(x)$ curve (i.e. a "vertical" effect). The magnitude of this vertical effect can be estimated as the difference between the responses of individuals of average age class \bar{x} in T treatment (exposed) versus C treatment (control): $\psi_T(\bar{x}) - \psi_C(\bar{x})$. Conversely, if the magnitude of individual responses varies with age, then the $\psi(x)$ curve will exhibit a different shape compared to the control curve. Assuming that the function $\psi(x)$ and its derivative are both monotonic, a change in shape is translated into changes in curvature, i.e. the degree of nonlinearity of $\psi(x)$ (a "nonlinear" effect). The degree of nonlinearity can be measured by the difference between the average response $\overline{\psi(x)}$ and the response of an individual of average age class $\psi(\bar{x})$. Thus the magnitude of a nonlinear effect can be estimated as the difference between treatment and control groups in their degree of nonlinearity: $\left(\overline{\psi_T(x)} - \psi_T(\bar{x})\right) - \left(\overline{\psi_C(x)} - \psi_C(\bar{x})\right)$.

In age-structured populations, variations in environmental factors that affect organisms' performance could exert both vertical and nonlinear effects, which may have different magnitude and even opposite sign. To analyze such scenarios, a decomposition procedure is needed to assess the relative contribution of vertical and nonlinear shifts to the total effect of the driving factor on the population value of maximal interaction strength. For

the purposes of our study, but easily adaptable to other scenarios, we developed the following decomposition procedure: $\overline{\psi_T(x)} - \overline{\psi_C(x)} = [\psi_T(\bar{x}) - \psi_C(\bar{x})] + \left[\left(\overline{\psi_T(x)} - \psi_T(\bar{x})\right) - \left(\overline{\psi_C(x)} - \psi_C(\bar{x})\right)\right]$, where $\psi(x)$ is the maximal interaction strength at age x , with subscripts C and T representing control and pesticide treatments, respectively. The magnitudes $\overline{\psi(x)} = n^{-1} \sum_{i=1}^n h_i^{-1}$ and $\psi(\bar{x}) = h_A^{-1}$ represent the average maximal interaction strength of the population over age classes i and the maximal interaction strength of an individual of average age class A , respectively. Parameter h^{-1} is the fitted value of the inverse of handling time obtained in each case. Symbol n represents the number of age classes considered. The left hand side of the above equation represents the reduction in the population (average) value of $\psi(x)$ as a consequence of pesticide exposure. The first term in the brackets on the right hand side represents the magnitude of the vertical effect (VE) and the second term in the brackets represents the magnitude of the nonlinear effect (NLE) of pesticide exposure on population maximal interaction strength. We computed 95 % confidence intervals by the percentile method for vertical and nonlinear effects from the bootstrapped datasets, as explained above. To express vertical and nonlinear effects in percentages, we calculated $\%VE = 100 \times |VE|/(|VE| + |NLE|)$ and $\%NLE = 100 \times |NLE|/(|VE| + |NLE|)$.

Results

Results from the generalized linear model analysis indicated that the feeding rate of *Daphnia* was significantly affected by the interaction among consumer age, food density and pollutant exposure (Table 3).

Using the Akaike information criterion, the Rogers model and the standard Holling type II functional response models were the equations that best described the data (Table 4). The Holling type II model behaved slightly better than the Rogers model in the controls, while the Rogers model behaved slightly better than the Holling type II model in the experimental treatments.

Table 3 Summary of generalized linear model analysis, using a negative binomial error distribution

Predictor	d.f.	Residual deviance	P
Null		17063.9	
Food	4	9921.2	< 0.0001
Age	4	1975.9	< 0.0001
Pollutant	1	918.3	< 0.0001
Food × age	16	651.6	< 0.0001
Food × pollutant	4	547.4	< 0.0001
Age × pollutant	4	394.7	< 0.0001
Food × age × pollutant	16	205.5	< 0.0001

We tested the incidence of food density, age of consumers, pollutant exposure and interactions on feeding rate. The residual deviance associated with the sequential inclusion of each predictor is presented. P values were estimated using the Chi square test

Table 4 Model selection results

Age (day)	Model	Control		Treatment	
		R^2	ΔAIC_c	R^2	ΔAIC_c
1	Standard Holling type II	0.972	0	0.949	0
	Holling type III	0.930	0.876	0.905	0.571
	Rogers random-predator	0.972	0.002	0.949	0.006
	Generalized Holling type III	0.973	1.750	0.948	1.828
	Holling type IV	0.973	1.782	0.947	1.838
3	Generalized Holling	0.969	4.165	0.946	4.113
	Standard Holling type II	0.982	0.094	0.967	0.027
	Holling type III	0.968	0.670	0.963	0.127
	Rogers random-predator	0.984	0	0.968	0
	Generalized Holling type III	0.985	1.678	0.974	1.579
5	Holling type IV	0.986	1.629	0.972	1.636
	Generalized Holling	0.940	5.257	0.922	4.841
	Standard Holling type II	0.966	0	0.964	0.001
	Holling type III	0.906	0.990	0.933	0.629
	Rogers random-predator	0.962	0.133	0.964	0
7	Generalized Holling type III	0.967	1.802	0.964	1.795
	Holling type IV	0.967	1.800	0.964	1.793
	Generalized Holling	0.951	4.453	0.950	4.300
	Standard Holling type II	0.978	0.051	0.962	0.336
	Holling type III	0.963	0.569	0.985	3.619
9	Rogers random-predator	0.979	0	0.973	0
	Generalized Holling type III	0.980	1.740	0.985	1.200
	Holling type IV	0.964	4.631	0.984	1.292
	Generalized Holling	0.935	5.083	0.900	5.274
	Standard Holling type II	0.986	0	0.989	0.016
9	Holling type III	0.947	1.331	0.968	1.032
	Rogers random-predator	0.948	1.302	0.989	0
	Generalized Holling type III	0.986	1.786	0.990	1.728
	Holling type IV	0.986	1.764	0.989	1.805
	Generalized Holling	0.957	5.084	0.965	5.241

Values of the R^2 and corrected scaled Akaike information criterion (ΔAIC_c) are given for each of the five tested models shown in Table 2, for control and pesticide treatments. In bold, the favored models

In the rest of the text we will only use the parameters obtained from the Rogers model since it accounts for decreases in food levels as occurred in our experiments. However, the results for the Holling type II model did not exhibit qualitative differences (Fig. 1). Parameter values obtained from fitting feeding rate data in each treatment to both the Rogers and Holling type-II model are given in Online Resource 1 (Tables S1 and S2).

Pesticide exposure significantly reduced average attack rate but did not exert any effect on its coefficient of variation. On the other hand, the pesticide significantly increased both the mean and coefficient of variation of handling time (Fig. 2, see also Fig. S1).

The shape of the relationship between maximal interaction strength and organism age, $\psi(x)$, was also affected by pesticide exposure. The function $\psi(x)$ was nearly linear in controls. In contrast, in the pesticide treatments the relationship was accelerating (Fig. 3, see also Fig. S2).

Finally, our decomposition procedure showed that pesticide exposure reduced $\psi(x)$ by 68 % (Rogers) and 65 % (Holling) at the population level due to a negative vertical effect, but this was counteracted by an increase in $\psi(x)$ of 32 % (Rogers) and 35 % (Holling) through a positive nonlinear effect (Figs. 4, S3). Thus, nonlinear

effects contributed to the total effect of pesticide by 47 % (Rogers) and 53 % (Holling).

Discussion

Within populations there is considerable variation in organisms' behavior and particularly in their foraging behavior (Kobler et al. 2009; Sweeney et al. 2013). The recognition of among-individual behavioral variation and the analysis of its evolutionary causes and system consequences at the population and community levels have emerged as a vigorous research field in the last decade (Wolf et al. 2007; Biro and Stamps 2010). Clearly, individuals of most species grow considerably in size during their lifespan, and this ontogenetic variation expressed in structured populations, explains a large part of the intra-population functional trait variation (Miller and Rudolf 2011; Persson and De Roos 2013). Theoretical studies have emphasized the importance of the scaling relationship between foraging rate and body size in the dynamics of populations and communities (Persson et al. 1998). Furthermore, recent empirical studies using isotopic analyses revealed that ontogenetic patterns of foraging behavior exhibit considerable inter-individual variation (Kim et al. 2012). Ontogenetic

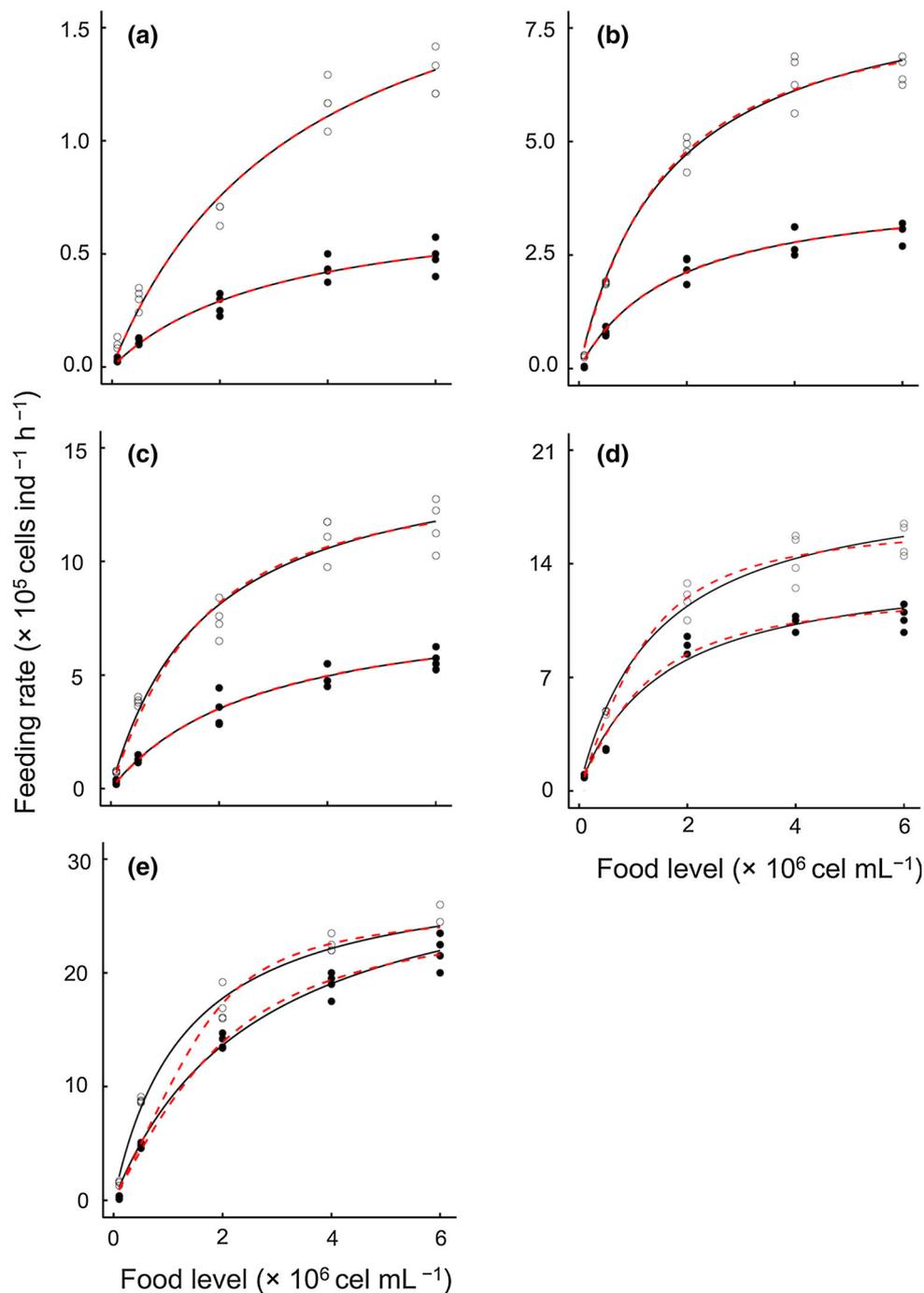


Fig. 1 Functional responses of *Daphnia ambigua* feeding on the microalga *Pseudokirchneriella subcapitata* across the consumers' ontogeny, for individuals of the following age classes: one day old (a), three days old (b), five days old (c), seven days old (d) and nine

days old (e). Experimental data were fitted to a Holling type II model (black continuous lines) and Rogers random-predator model (red dashed lines). Open and filled circles represent control and pesticide exposure treatments, respectively

variation in foraging behavior is often associated with differential capabilities of consumers to capture, manipulate and ingest their prey, but also to micro-habitat shifts or differential habitat use during ontogeny, leading to an ontogenetic niche shift (Ramos-Jiliberto et al. 2011 and references therein) that is known to

influence population and community dynamics (Polis et al. 1996; Takimoto 2003).

The functioning of multispecies food webs is affected by interaction strengths among species, which are governed by foraging rates at the population level, that is, averaged over individuals. Nevertheless, whenever

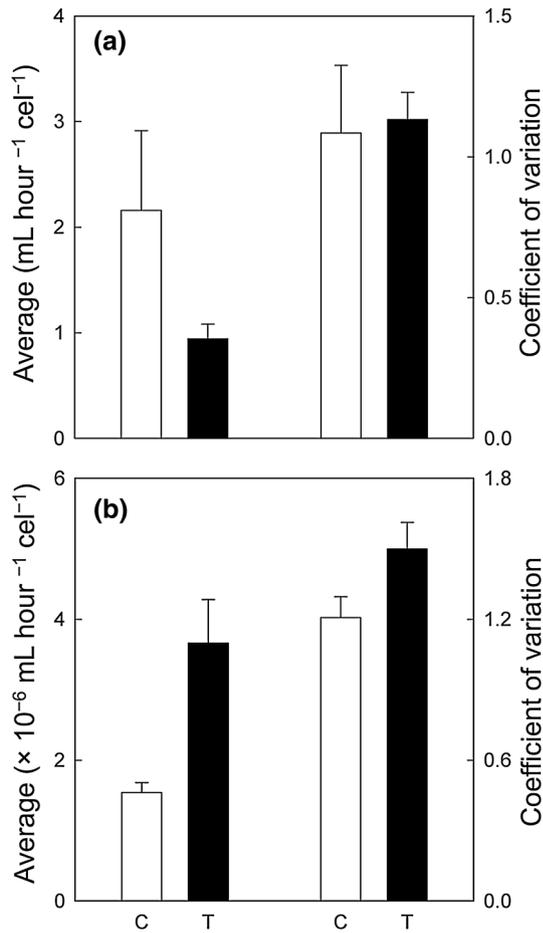


Fig. 2 Average and coefficient of variation of the functional response parameters attack rate (a) and handling time (b), both in control (C, white bars) and pesticide treatment (T, black bars). Parameter values were obtained by fitting the data to the Rogers random-predator model

interaction strength scales nonlinearly with some relevant behavioral parameters, both the mean and variance of parameter values determine population interaction strength (Gibert and Brassil 2014). This effect is known as Jensen's inequality (Jensen 1906; Ruel and Ayres 1999), which is nowadays recognized as an important link between individual trait variation and several ecological processes such as thermal preferences (Martin and Huey 2008), predator reproductive rates (Okuyama 2013) and population dynamics (Okuyama 2008; Estay and Lima 2010).

In this study we analyzed how a model stressor (the pesticide methamidophos) modifies the population level predator–prey interaction strength between *Daphnia* and its food by shifting the values of individual-level behavioral parameters that govern consumer feeding rates. We focused on the ontogenetic determinants of inter-individual variation in interaction strength and decomposed the effect of pollutant exposure on population interaction strength values into contributions from vertical and nonlinear effects. We showed that nearly 50 % of the effect of the pesticide on predator–

prey interaction strength was explained by changes in the distribution of effect sizes over age (i.e. nonlinear effects).

We obtained differences in feeding rates up to 40-fold between older and younger individuals. This explains our difficulties in maintaining the same container size and incubation time across the different consumer ages, which could lead to some error in our estimations. For example, it has been suggested that reductions in container size may affect filtering rates by increasing surface-to-volume ratios and permitting dilute food suspensions to concentrate on surfaces (Horton et al. 1979; Peters and Downing 1984; Enserink et al. 1990). In our case, the differences in medium volume across treatments were not too large, and we attempted to reduce alga settling by gently shaking the containers during trials. Although we made efforts to prevent large errors in estimating feeding rates, our quantitative results should be interpreted with caution. Another limitation of this study is that it was conducted with single strains of *D. ambigua* and *P. subcapitata*. However, our decomposition analysis is fully valid, and we expect to use other strains and species in future studies.

Our results give empirical support to the hypothesis that inter-individual variation in feeding behavior during ontogeny affects species interactions, which are known to govern community dynamics (Werner and Gilliam 1984; Nakazawa 2015). In particular, we showed that ontogenetic changes in feeding rates, without invoking diet shift, determine interaction strength among populations. In this way we expand the common view that ontogenetic changes in feeding behavior drive community dynamics solely through niche shifts. Furthermore, we highlight that the effects of perturbations in general, and pollutants in particular, on community functioning can only be understood by considering the changes in the frequency distribution of individual parameter values, and not just considering effects on the mean.

Pollution currently represents one of the main threats to biodiversity (MEA 2005), affecting the vast majority of ecosystems on earth (Groom et al. 2006). The effects of pollutants on ecological systems are being studied at every level of biological organization, from biomolecules to communities and ecosystems. Nevertheless, to develop effective management actions the effects of pollutant must be understood at the highest levels of organization, especially at the population and community levels (Hope 2006; Kelly et al. 2007). In spite of the obvious need to understand how pollutants are affecting natural populations and communities as well as how these impairments could be avoided or minimized, ecological theory has not shown enough progress in this direction, with relatively few successful attempts to filling this gap, especially at the community level (Garay-Narváez et al. 2013, 2014).

Predator–prey interaction strength is known to be a strong determinant of community dynamics (McCann et al. 1998; Kokkoris et al. 1999), and therefore any

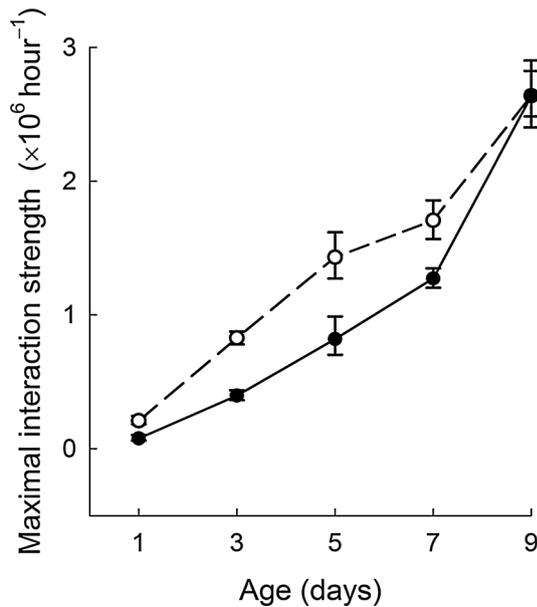


Fig. 3 Relationship between maximal interaction strength, given by the inverse of handling time (mean \pm 95 % confidence intervals), and age of the consumer. Parameter values were obtained by fitting the data to the Rogers random-predator model. Filled circles pesticide exposure treatments. Open circles controls

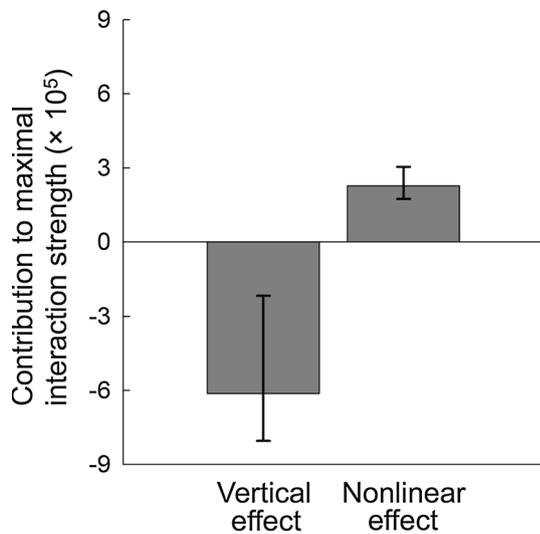


Fig. 4 Decomposition of changes in population maximal interaction strength driven by pesticide exposure into contributions from vertical and nonlinear effects (mean \pm 95 % confidence intervals). Functional response parameter values were obtained by fitting the data to the Rogers random-predator model

alteration of its values due to exposure to stressors may affect community stability and species persistence. Methamidophos, as other organophosphate pesticides, is an acetylcholinesterase (AChE) inhibitor. AChE inhibition may cause disturbances in transmission across cholinergic synapses in both central and peripheral nerves, producing a synaptic block of neuromuscular junctions which may finally end in muscle spasms (Ware 1983; Walker 2009). The feeding process in *Daphnia*,

which requires the movement of appendages and the coordination of nervous system, is prone to be affected by AChE inhibition (Fernández-Casalderrey et al. 1994; Villarroel et al. 1998, 1999; Agatz et al. 2013), which could explain why *Daphnia* functional response parameters are sensitive to methamidophos exposure. Nevertheless, organisms of different age and size are not equally sensitive to any given toxicant. Thus, in a structured population, differential responses of organisms to stressor exposure lead to different shifts in feeding parameters, which in turn could lead to a change in the elevation and curvature of the relation between interaction strength and age. As a consequence of Jensen's inequality, curvature shifts could either reinforce or dampen the effect of a stressor on the predator foraging rate at the population level, depending on the shape of the interaction strength/age relation. In our study, curvature shift (nonlinear effect) dampened the effect of elevation shift (vertical effect), the latter tending towards decreasing maximum population interaction strength. This means that the effect of pesticide exposure could be overestimated if shifts in curvature are not addressed.

It is well known that many chemical pollutants exert effects of different magnitude on organisms of different age or size (Hutchinson et al. 1998; Vives et al. 2005). In most cases toxicants affect younger individuals in a population more strongly, which is explained by higher bioconcentration of chemicals in smaller organisms. This is because bioconcentration is inversely proportional to body mass (Hendriks 1995; Kooijman 2000) and directly proportional to body lipid content, which decreases during *Daphnia* development (Bychek and Gushchina 1999). However, in some cases this general pattern is modified during development via changes in the processes of uptake, distribution, biotransformation and elimination of toxicants from the body (Hamm et al. 2001; Vives et al. 2005; Preuss et al. 2008). For example, Collyard et al. (1994) observed that younger organisms of *Hyalella azteca* were less sensitive to an organophosphate pesticide than older ones, and attributed their results to a less well-developed system of young amphipods for activating the parent phosphorothioate molecule to its toxic oxon derivative. However, our results indicate that individuals of intermediate stages of the life cycle are more affected by the pesticide than those of earlier or later stages. To our knowledge there is no clear or unique explanation for this pattern, but some prior insights may help to understand the mechanisms behind our results. It has been shown that cladocerans are more sensitive to pesticides during their post-molt periods, possibly due to a higher uptake rate (Gliwicz and Sieniawska 1986). This suggests that *Daphnia* may be more harmed by methamidophos before reaching their first reproductive instar, since more molts occur prior to this stage.

It is likely that different species within a community will exhibit different age-specific changes in several functional trait values driven by stress exposure. The

consequences of such effects are not easy to predict without conducting specific experiments. It is nevertheless conceivable that such species-specific shifts in the relation between trait values and age could lead to significant modification of the shape of the frequency distribution of trait values in the community, with unpredictable effects on community dynamics and stability. In the case of interaction strength, deviations from the few-strong-many-weak interaction strength pattern are expected by exposure to stressors, giving rise to interesting and urgent questions for understanding and managing wild ecosystems in the Anthropocene. Future studies could project the consequences of our experimental findings at the population and community levels using dynamic modeling tools.

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References

- Abrams PA (2001) Describing and quantifying interspecific interactions: a commentary on recent approaches. *Oikos* 94:209–218
- Agatz A, Cole TA, Preuss TG, Zimmer E, Brown CD (2013) Feeding inhibition explains effects of imidacloprid on the growth, maturation, reproduction, and survival of *Daphnia magna*. *Environ Sci Technol* 47:2909–2917
- Berlow EL, Neutel A-M, Cohen JE, de Ruiter PC, Ebenman B, Emmerson M, Fox JW, Jansen VAA, Iwan Jones J, Kokkoris GD, Logofet DO, McKane AJ, Montoya JM, Petchey O (2004) Interaction strengths in food webs: issues and opportunities. *J Anim Ecol* 73:585–598
- Biro PA, Stamps JA (2010) Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends Ecol Evol* 25:653–659
- Bolnick DI, Amarasekare P, Araújo MS, Bürger R, Levine JM, Novak M, Rudolf VH, Schreiber SJ, Urban MC, Vasseur DA (2011) Why intraspecific trait variation matters in community ecology. *Trends Ecol Evol* 26:183–192
- Burnham K, Anderson D (2004) Multimodel inference: understanding AIC and BIC in model selection. *Sociol Method Res* 33:261–304
- Bychek EA, Gushchina IA (1999) Age-dependent changes of lipid composition in *Daphnia magna*. *Biochemi Moscow* 64:543–545
- Collyard SA, Ankley GT, Hoke RA, Goldenstein T (1994) Influence of age on the relative sensitivity of *Hyaella azteca* to diazinon, alkylphenol ethoxylates, copper, cadmium, and zinc. *Arch Environ Con Tox* 26:110–113
- Day K, Kaushik NK (1987) Short-term exposure of zooplankton to the synthetic pyrethroid fenvalerate, and its effects on rates of filtration and assimilation of the alga, *Chlamydomonas reinhardtii*. *Arch Environ Con Tox* 16:423–432
- Enserink L, Luttmer W, Maas-Diepeveen H (1990) Reproductive strategy of *Daphnia magna* affects the sensitivity of its progeny in acute toxicity tests. *Aquat Toxicol* 17:15–26
- Estay S, Lima M (2010) Combined effect of ENSO and SAM on the population dynamics of the invasive yellow jacket wasp in central Chile. *Popul Ecol* 52:289–294
- Fernández-Casalderrey A, Ferrando MD, Andreu-Moliner E (1994) Effect of sublethal concentrations of pesticides on the feeding behaviour of *Daphnia magna*. *Ecotoxicol Environ Safe* 27:82–89
- Fussmann GF, Weithoff G, Yoshida T (2005) A direct, experimental test of resource vs. consumer dependence. *Ecology* 86:2924–2930
- Garay-Narváez L, Arim M, Flores JD, Ramos-Jiliberto R (2013) The more polluted the environment, the more important biodiversity is for food web stability. *Oikos* 122:1247–1253
- Garay-Narváez L, Arim M, Flores JD, Ramos-Jiliberto R (2014) Food web modularity and biodiversity promote species persistence in polluted environments. *Oikos* 123:583–588
- García de la Parra LM, Bautista-Covarrubias JC, Rivera-de la Rosa N, Betancourt-Lozano M, Guilhermino L (2006) Effects of methamidophos on acetylcholinesterase activity, behavior, and feeding rate of the white shrimp (*Litopenaeus vannamei*). *Ecotoxicol Environ Safe* 65:372–380
- Gibert JP, Brassil CE (2014) Individual phenotypic variation reduces interaction strengths in a consumer–resource system. *Ecol Evol* 4:3703–3713
- Gibert JP, Dell AI, DeLong JP, Pawar S (2015) Scaling-up trait variation from individuals to ecosystems. *Adv Ecol Res* 52:1–17
- Gliwicz MZ, Sieniawska A (1986) Filtering activity of *Daphnia* in low concentrations of a pesticide. *Limnol Oceanogr* 31:1132–1138
- Groom MJ, Meffe GK, Carroll CR (2006) Principles of conservation biology. Third Edition, Sinauer
- Hamm JT, Wilson BW, Hinton DE (2001) Increasing uptake and bioactivation with development positively modulate diazinon toxicity in early life stage medaka (*Oryzias latipes*). *Toxicol Sci* 61:304–313
- Hanazato T (1991) Effects of repeated applications of Carbaryl on zooplankton communities in experimental ponds with or without the predator *Chaoborus*. *Environ Pollut* 74:309–324
- Hendriks AJ (1995) Modeling nonequilibrium concentrations of micro-contaminants in organisms—comparative kinetics as a function of species size and octanol water partitioning. *Chemosphere* 30:265–292
- Hope BK (2006) An examination of ecological risk assessment and management practices. *Environ Int* 32:983–995
- Horton PA, Rowan M, Webster KE, Peters RH (1979) Browsing and grazing by cladoceran filter feeders. *Can J Zool* 57:206–212
- Hughes AR, Hanley TC, Orozco NP, Zerebecki RA (2015) Consumer trait variation influences tritrophic interactions in salt marsh communities. *Ecol Evol* 5:2659–2672
- Hussain MA (1987) Anticholinesterase properties of methamidophos and acephate in insects and mammals. *B Environ Contam Toxicol* 38:131–138
- Hutchinson TH, Solbe J, Klopper-Sams PJ (1998) Analysis of the ecotoxic aquatic toxicity (EAT) database III—comparative toxicity of chemical substances to different life stages of aquatic organisms. *Chemosphere* 36:129–142
- Jensen JLWV (1906) Sur le fonctions convexes et les inégalités entre les valeurs moyennes. *Acta Mat* 30:175–193
- Jeschke JM, Kopp M, Tollrian R (2002) Predator functional responses: discriminating between handling and digesting prey. *Ecol Monogr* 72:95–112
- Kelly BC, Ikonomou MG, Blair DJ, Morin AE, Gobas FAPC (2007) Food web-specific biomagnification of persistent organic pollutants. *Science* 317:236–239
- Kilham SS, Keeger DA, Lynn SG, Goulden CE, Herrera DL (1998) COMBO: a defined freshwater culture medium for algae and zooplankton. *Hydrobiologia* 377:147–159
- Kim SL, Tinker MT, Estes JA, Koch PL (2012) Ontogenetic and among-individual variation in foraging strategies of northeast Pacific white Sharks based on stable isotope analysis. *PLoS One* 7:e45068
- Kobler A, Klefoth T, Mehner T, Arlinghaus R (2009) Coexistence of behavioural types in an aquatic top predator: a response to resource limitation? *Oecologia* 161:837–847

- Kokkoris GD, Troumbis AY, Lawton JH (1999) Patterns of species interaction strength in assembled theoretical competition communities. *Ecol Lett* 2:70–74
- Kooijman SALM (2000) Dynamic energy and mass budgets in biological systems. Cambridge University Press, Cambridge
- Kooijman SALM, Metz JAJ (1984) On the dynamics of chemical stressed populations: the deduction of population consequences from effects on individuals. *Ecotoxicol Environ Safe* 8:254–274
- Laska MS, Wootton JT (1998) Theoretical concepts and empirical approaches to measuring interaction strength. *Ecology* 79:461–476
- Lin K, Zhou S, Xu C, Liu W (2006) Enantiomeric resolution and biotoxicity of methamidophos. *J Agric Food Chem* 54:8134–8138
- Malato S, Blanco J, Richter C, Milow B, Maldonado MI (1999) Solar photocatalytic mineralization of commercial pesticides: methamidophos. *Chemosphere* 38:1145–1156
- Manly BFJ (2007) Randomization, bootstrap and Monte Carlo methods in biology, 3rd edn. Chapman & Hall/CRC, Boca Raton
- Martin TL, Huey RB (2008) Why “suboptimal” is optimal: Jensen’s inequality and ectotherm thermal preferences. *Am Nat* 171:E102–E118
- McCann K, Hastings A, Huxel GR (1998) Weak trophic interactions and the balance of nature. *Nature* 395:794–797
- Millennium Ecosystem Assessment (MEA) (2005) Ecosystems and human well-being: biodiversity synthesis. World Resources Institute, Washington
- Miller TEX, Rudolf VHW (2011) Thinking inside the box: community-level consequences of stage-structured populations. *Trends Ecol Evol* 26:457–466
- Nakazawa T (2015) Ontogenetic niche shifts matter in community ecology: a review and future perspectives. *Popul Ecol* 57:347–354
- OECD (2004) OECD guidelines for testing of chemicals. Guideline 202: *Daphnia* sp., Acute immobilisation test, adopted April 2004
- Okuyama T (2008) Individual behavioral variation in predator–prey models. *Ecol Res* 23:665–671
- Okuyama T (2013) Consequences of variation in foraging success among predators on numerical response. *Ecol Evol* 3:4039–4043
- Pawar S, Dell AI, Savage VM (2015) From metabolic constraints on individuals to the eco-evolutionary dynamics of ecosystems. In: Belgrano A, Woodward G, Jacob U (eds) Aquatic functional biodiversity: an eco-evolutionary approach. Elsevier, Amsterdam
- Persson L, De Roos AM (2013) Symmetry breaking in ecological systems through different energetic efficiencies of juveniles and adults. *Ecology* 94:1487–1498
- Persson L, Leonardsson K, de Roos AM, Gyllenberg M, Christensen B (1998) Ontogenetic scaling of foraging rates and the dynamics of a size-structured consumer–resource model. *Theor Popul Biol* 54:270–293
- Peters RH (1984) Methods for the study of feeding, grazing and assimilation by zooplankton. In: Downing JA, Rigler FH (eds) A manual on methods for the assessment of secondary production in freshwaters. IBP Handbook 17. Blackwell, Hoboken, pp 336–412
- Peters RH, Downing JA (1984) Empirical analysis of zooplankton filtering and feeding rates. *Limnol Oceanogr* 29:763–784
- Polis GA (1984) Age structure component of niche width and intraspecific resource partitioning—can age-groups function as ecological species. *Am Nat* 123:541–564
- Polis GA, Holt RD, Menge BA, Winemiller KO (1996) Time, space, and life history: influences on food webs. In: Winemiller KO, Polis GA (eds) Food Webs: Integration of Patterns and Dynamics. Chapman and Hall, New York, pp 435–460
- Preuss TG, Telscher M, Ratte HT (2008) Life stage-dependent bioconcentration of nonylphenol isomer in *Daphnia magna*. *Environ Pollut* 156:1211–1217
- Ramos-Jiliberto R, Valdovinos FS, Arias J, Alcaraz C, García-Berthou E (2011) A network-based approach to the analysis of ontogenetic diet shifts: an example with an endangered, small-sized fish. *Ecol Complex* 8:123–129
- Rudolf VHW, Rasmussen NL (2013) Population structure determines functional differences among species and ecosystem processes. *Nat Commun* 4:2318
- Ruel JJ, Ayres MP (1999) Jensen’s inequality predicts effects of environmental variation. *Trends Ecol Evol* 14:361–366
- Sala E, Graham MH (2002) Community-wide distribution of predator–prey interaction strength in kelp forests. *Proc Natl Acad Sci* 99:3678–3683
- Schreiber S, Bürger R, Bolnick DI (2011) The community effects of phenotypic and genetic variation within a predator population. *Ecology* 92:1582–1593
- Sweeney K, Cusack B, Armagost F, O’Brien T, Keiser CN, Pruitt JN (2013) Predator and prey activity levels jointly influence the outcome of long-term foraging bouts. *Behav Ecol* 24:1205–1210
- Takahashi H, Hanazato T (2007) Synergistic effects of food shortage and an insecticide on a *Daphnia* population: rapid decline of food density at the peak of population density reduces tolerance to the chemical and induces a large population crash. *Limnology* 8:45–51
- Takimoto G (2003) Adaptive plasticity in ontogenetic niche shifts stabilizes consumer–resource dynamics. *Am Nat* 162:93–109
- Tomin C (1994) The pesticide manual, a world compendium, 10th edn. British Crop Protection Council, Croydon
- Vázquez DP, Ramos-Jiliberto R, Urbani P, Valdovinos FS (2015) A conceptual framework for studying the strength of plant–animal mutualistic interactions. *Ecol Lett* 18:385–400
- Villarreal MJ, Ferrando MD, Andreu E (1998) Toxic anorexia as a sensitive endpoint in *Daphnia magna*. *J Environ Sci Health B* 33:151–160
- Villarreal MJ, Ferrando MD, Sancho E, Andreu E (1999) *Daphnia magna* feeding behavior after exposure to tetradifon and recovery from intoxication. *Ecotox Environ Safe* 44:40–46
- Violle C, Enquist BJ, McGill BJ, Jiang L, Albert CH, Hulshof C, Jung V, Messier J (2012) The return of the variance: intraspecific variability in community ecology. *Trends Ecol Evol* 27:244–252
- Vives I, Grimalt JO, Ventura M, Catalan J, Rosseland BO (2005) Age dependence of the accumulation of organochlorine pollutants in brown trout (*Salmo trutta*) from a remote high mountain lake (Redó, Pyrenees). *Environ Pollut* 133:343–350
- Walker CH (2009) Organic pollutants: an ecotoxicological perspective, 2nd edn. CRC Press/Taylor & Francis, Boca Raton, p 203
- Ware GW (1983) Pesticides, theory and applications. Freeman and Company, San Francisco
- Werner EE, Gilliam JF (1984) The ontogenetic niche and species interactions in size-structured populations. *Annu Rev Ecol Syst* 15:393–425
- WHO (1993) Methamidophos health and safety guide no 79. World Health Organization, Geneva
- Wolf M, van Doorn GS, Leimar O, Weissing FJ (2007) Life-history trade-offs favour the evolution of animal personalities. *Nature* 447:581–584
- Wootton JT (1997) Estimates and tests of per capita interaction strength: diet, abundance, and impact of intertidally foraging birds. *Ecol Monogr* 67:45–64
- Wootton JT, Emmerson M (2005) Measurement of interaction strength in nature. *Annu Rev Ecol Syst* 36:419–444
- Yen JH, Lin KH, Wang YS (2000) Potential of the insecticides acephate and methamidophos to contaminate ground water. *Ecotoxicol Environ Safe B* 45:79–86
- Yodzis P (1989) Introduction to theoretical ecology. Harper and Row, NY